Claims 1-116 are pending.

Claims 13, 23, 79, 109, 112 and 115 are amended to correct obvious typographical and spelling errors and to produce grammatical clarity. In particular claim 23 is amended to add the inadvertently omitted noun —acid— for grammatical clarity. The amendment finds basis at page 52, line 15, of the specification. Claim 79 is amended to replace the verb "add" with the verb —adding— for grammatical clarity. Claims 109, 112 and 115 are amended to remove the inadvertently added phrase "is tumor" for grammatical clarity.

The specification is amended to correct obvious typographical and spelling errors and to produce grammatical clarity. In particular the amendments to the paragraphs on page 68, line 27, through page 69, line 11, and page 71, lines 12-27, of the specification replace chemical compound name "a-amino isobutyric acid" with chemical compound name —*α*-amino isobutyric acid— for proper nomenclature.

No new matter has been added.

Included as an attachment is a marked-up version of the specification paragraphs and claims, per 37 CFR §1.121.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Edwin Madison *et.al.*
Serial No.:10/099,700
Filed: March 13, 2002

For: *NUCLEIC ACID MOLECULES ENCODING A TRANSMEMBRANE SERINE PROTEASE 7, THE ENCODED POLYPEPTIDES AND METHODS BASED THEREON*

Confirmation No.: 4309
Art Unit: 1645
Examiner: Unassigned

ATTACHMENT TO THE PRELIMINARY AMENDMENT
MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph on page 1, lines 17-29, as follows:

Cancer, which is a leading cause of death in the United States, is characterized by an increase in the number of abnormal neoplastic cells, which proliferate to form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells that metastasize via the blood or lymphatic system to regional lymph nodes and to distant sites. Among the hallmarks of cancer is a breakdown in the communication among tumor cells and their environment. Normal cells do not divide in the absence of stimulatory signals and cease dividing in the presence of inhibitory signals. Growth-stimulatory and growth-inhibitory signals, are routinely exchanged between cells within a tissue. In a cancerous, or [neoplastic,] <u>neoplastic</u> state, a cell acquires the ability to "override" these signals and to proliferate under conditions in which normal cells do not grow.

Please amend the paragraph on page 2, lines 19-25, as follows:

A class of extracellular matrix degrading enzymes [have] <u>has</u> been implicated in tumor invasion. Among these are the matrix metalloproteinases (MMP). For example, the production of the matrix metalloproteinase stromelysin is associated with malignant tumors with metastatic potential (see, *e.g.*,

McDonnell *et al.* (1990) *Smnrs. in Cancer Biology 1*:107-115; McDonnell *et al.* (1990) *Cancer and Metastasis Reviews 9*:309-319)

Please amend the paragraph on page 3, lines 4-13, as follows:

In addition to the MMPs, serine proteases have been implicated in neoplastic disease progression. Most serine proteases, which are either secreted enzymes or are sequestered in cytoplasmic storage organelles, have roles in blood coagulation, wound healing, digestion, immune responses and tumor invasion and metastasis. A class of cell [of] surface proteins designated type II transmembrane serine proteases, which are membrane-anchored proteins with additional extracellular domains, has been identified. As cell surface proteins, they are positioned to play a role in intracellular signal transduction and in mediating cell surface proteolytic events.

Please amend the paragraph beginning on page 6, line 23 through page 7, line 8, as follows:

Assays for identifying effectors, such as small molecules and other conditions, that modulate the activation, expression or activity of MTSP7 are also provided herein. In exemplary assays, the [affects] effects of test compounds on the ability of a protease domain of MTSP7 to proteolytically cleave a known substrate, typically a fluorescently, chromogenically or otherwise detectably labeled substrate, are assessed. Agents, generally compounds, particularly small molecules, that modulate the activity of the protease domain are candidate compounds for modulating the activity of the MTSP7. The protease domains can also be used to produce protease-specific antibodies. The protease domains provided herein include, but are not limited to, the single chain region having an N-terminus at the cleavage site for activation of the zymogen, through the C-terminus, or C-terminal truncated portions thereof that exhibit proteolytic activity as a single-chain polypeptide in *vitro* proteolysis assays, of any MTSP family member, including MTSP7, generally from a mammal, including human, that, for example, is expressed in tumor cells at different levels from non-tumor cells.

Please amend the paragraph on page 7, lines 9-19, as follows:

Nucleic acid molecules encoding the proteins and protease domains are also provided. The nucleic acid and amino acid sequences of an exemplary full length MTSP7 are set forth in SEQ ID Nos. 15 and 16, and the protease domain is set forth in SEQ ID No. 17 and 18. Nucleic acid molecules that encode a single-chain protease domain or catalytically active portion thereof and also those that encode the full-length MTSP7 are provided. Also provided are nucleic acid molecules that hybridize to such MTSP7-encoding nucleic acid along their full length and encode the protease domain or portion thereof[are provided]. Hybridization is generally effected under conditions of at least low, generally at least moderate, and often high stringency.

Please amend the paragraph on page 9, lines 10-17, as follows:

In certain embodiments, the MTSP7 polypeptide is detectable in a body fluid at a level that differs from its level in body fluids in a subject not having a tumor. In other embodiments, the polypeptide is present in a tumor; and a substrate or cofactor for the polypeptide is expressed at levels that differ from its level of expression in a non-tumor cell in the same type of tissue. In other embodiments, the substantially purified [the] level of expression and/or activity of the MTSP7 polypeptide in tumor cells differs from its level of expression and/or activity in non-tumor cells.

Please amend the paragraph on page 11, lines 18-28, as follows:

MTSP7 proteins, including, but not limited [including] <u>to</u> splice variants thereof, and nucleic acids encoding MTSPs, and domains, derivatives and analogs thereof are provided herein. Single chain protease domains that have an N-terminus generated by activation of the zymogen form of MTSP7 are also provided. The cleavage site for the protease domain is at amino acid I_{206} (R↓IVQG). The Cys residues at positions C_{186}-C_{313}, which links protease domain to another domain, C_{233}-C_{249}, C_{358}-C_{374} and C_{385}-C_{413} form disulfide bonds, so that upon cleavage the resulting polypeptide is a two chain molecule. Hence C_{313} is a free Cys in the protease domain, which can also be provided as a two

chain molecule. It is shown herein, however, that the single chain form is proteolytically active.

Please amend the paragraph on page 13, lines 16-22, as follows:

Also provided are articles of manufacture that contain MTSP7 protein and protease domains of MTSP7 in single chain forms or activated forms. The articles contain a) packaging material; b) the polypeptide (or encoding nucleic acid), particularly the single chain protease domain thereof; and c) a label indicating that the article is for [using ins] use in assays for identifying modulators of the activities of an MTSP7 protein [is provided herein].

Please amend the paragraph on page 13, lines 23-30, as follows:

Conjugates containing a) a MTSP7 protein or protease domain in single chain [from] form; and b) a targeting agent linked to the MTSP directly or via a linker, wherein the agent facilitates: i) affinity isolation or purification of the conjugate; ii) attachment of the conjugate to a surface; iii) detection of the conjugate; or iv) targeted delivery to a selected tissue or cell, [is] are provided herein. The conjugate can contain a plurality of agents linked thereto. The conjugate can be a chemical conjugate; and it can be a fusion protein.

Please amend the paragraph on page 16, lines 4-14, as follows:

Also provided are methods of identifying a compound that binds to the single-chain or two-chain form of MTSP7, by contacting a test compound with [a] both the forms; determining to which form the compound binds; and if it binds to a form of MTSP7, further determining whether the compound has at least one of the following properties:

(i) inhibits activation of the single-chain zymogen form of MTSP7;

(ii) inhibits activity of the two-chain or single-chain form; and

(iii) inhibits dimerization of the protein.

The forms can be full length or the protease domain resulting from cleavage at the RI activation site.

Please amend the paragraph on page 17, line 27, through page 18, line 16, as follows:

As used herein, "transmembrane serine protease (MTSP)" refers to a family of transmembrane serine proteases that share common structural features as described herein (see, also Hooper *et al.* (2001) *J. Biol. Chem.276*:857-860). Thus, reference, for example, to "MTSP" encompasses all proteins encoded by the MTSP gene family, including but are not limited to: MTSP1, MTSP3, MTSP4, MTSP6, MTSP7 or an equivalent molecule obtained from any other source or that has been prepared synthetically or that exhibits the same activity. Other MTSPs include, but are not limited to, corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4. The term also [encompass] encompasses MTSPs with amino acid substitutions that do not substantially alter activity of each member, and also encompasses splice variants thereof. Suitable conservative substitutions of amino acids are known to those of skill in this art and can be made generally without altering the enzymatic activity of the resulting molecule or without eliminating. Of particular interest are MTSPs of mammalian, including human, origin. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, *e.g.*, Watson *et al. Molecular Biology of the Gene*, 4th Edition, 1987, The Bejacmin/Cummings Pub. co., p.224).

Please amend the paragraph on page 18, lines 22-31, as follows:

As used herein, a "protease domain of an MTSP" refers to the protease domain of MTSP that is located within the extracellular domain of a MTSP and exhibits proteolytic activity. It includes at least the smallest fragment thereof that acts catalytically as a single chain form. Hence it is at least the minimal portion of the extracellular domain that exhibits proteolytic activity as assessed by standard [assays] *in vitro* assays. Those of skill in this art recognize that such protease domain is the portion of the protease that is structurally

equivalent to the trypsin or chymotrypsin fold. [Contemplatd] Contemplated herein are such protease domains and catalytically active portions thereof.

Please amend the paragraph on page 19, lines 1-21, as follows:

The MTSP7 protein, with the protease domains indicated, is illustrated in [Figure 1,] Figure 1. Smaller portions thereof that retain protease activity are contemplated. The protease domains from MTSPs vary in size and constitution, including insertions and deletions in surface loops. They retain conserved structure, including at least one of the active site triad (see, *e.g.*, the catalytic triad of the MTSP in SEQ ID No. 16 is H_{248}, D_{293}, S_{389}), primary specificity pocket, oxyanion hole and/or other features of serine protease domains of proteases. Thus, for purposes herein, the protease domain is a portion of a MTSP, as defined herein, and is homologous to a domain of other MTSPs, such as corin, enterpeptidase, human airway trypsin-like protease (HAT), MTSP1, TMPRSS2, and TMPRSS4, which have been previously identified; it was not recognized, however, that an isolated single chain form of the protease domain could function proteolytically in *in vitro* assays. As with the larger class of enzymes of the chymotrypsin (S1) fold (see, *e.g.*, Internet accessible MEROPS data base), the MTSPs protease domains share a high degree of amino acid sequence identity. The His, Asp and Ser residues necessary for activity are present in conserved motifs. The activation site, which results in the N-terminus of the second chain in the two chain [forms] forms, [is] has a conserved motif and readily can be identified (see, *e.g.*, amino acids 2-6-208).

Please amend the paragraph on page 23, line 27 through page 24, line 2, as follows:

For the protease domains, residues at the N-terminus can be critical for activity. It is shown herein that the protease domain of the single chain form of the MTSP7 protease is catalytically active. Hence the protease domain will require the N-terminal amino acids; the [c-terminus] C-terminus portion can be truncated. The amount that can be removed can be determined empirically by

testing the protein for protease activity in an *in vitro* [assays] assay that assesses catalytic cleavage.

Please amend the paragraph on page 27, line 26, through page 28, line 9, as follows:

As used herein, heterologous DNA is DNA that encodes RNA and proteins that are not normally produced *in vivo* by the cell in which it is expressed or that mediates or encodes mediators that alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes. Heterologous DNA can also be referred to as foreign DNA. Any DNA that one of skill in the art would recognize or consider as heterologous or foreign to the cell in which it is expressed is herein encompassed by heterologous DNA. Examples of heterologous DNA include, but are not limited to, DNA that encodes traceable marker proteins, such as a protein that confers drug resistance, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies. Antibodies that are encoded by heterologous DNA can be secreted or expressed on the surface of the cell in which the heterologous DNA has been introduced.

Please amend the paragraph on page 29, lines 10-20, as follows:

As used herein, nucleic acids include DNA, RNA, dsRNA and [anlog] analogs thereof, including protein nucleic acids (PNA) and mixture thereof. Nucleic acids can be single or double stranded. When referring to probes or primers, optionally labeled, with a detectable label, such as a fluorescent or radiolabel, single-stranded molecules are contemplated. Such molecules are typically of a length such that target is statistically unique or of low copy number (typically less than 5, generally less than 3) for probing or priming a library. Generally a probe or primer contains at least 14, 16 or 30 contiguous of sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

Please replace the paragraph on page 30, lines 7-28, with the following paragraph:

As used herein, operative linkage of heterologous DNA to regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences refers to the relationship between such DNA and such sequences of nucleotides. For example, operative linkage of heterologous DNA to a promoter refers to the physical relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA in reading frame. Thus, as used herein, operatively linked or operationally associated refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or *in vitro* transcription, it can be necessary to remove, add or alter 5′ untranslated portions of the clones to eliminate extra, [potential] potentially inappropriate alternative translation initiation (*i.e.*, start) codons or other sequences that can interfere with or reduce expression, either at the level of transcription or translation.

Please amend Table 1 on page 32, lines 1-21 as follows:

TABLE 1

Original residue	Conservative substitution
Ala (A)	Gly; Ser, Abu
Arg (R)	Lys, orn
Asn (N)	Gln; His
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
Gly (G)	Ala; Pro
His (H)	Asn; Gln
Ile (I)	Leu; Val; Met; Nle; Nva

Original residue	Conservative substitution
Leu (L)	Ile; Val; Met; Nle; Nv
Lys (K)	Arg; Gln; Glu
Met (M)	Leu; Tyr; Ile; NLe Val
[Ornitine] Ornithine	Lys; Arg
Phe (F)	Met; Leu; Tyr
Ser (S)	Thr.
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu; Met; Nle; Nv

Please amend the paragraph on page 33, line 26 through page 34, line 2, as follows:

As used herein, antibody refers to an immunoglobulin, whether natural or partially or wholly synthetically produced, including any derivative thereof that retains the specific binding ability of the antibody. Hence antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin binding domain. Antibodies include members of any immunoglobulin [claims] chains, including IgG, IgM, IgA, IgD and IgE.

Please replace the paragraph on page 34, lines 3-10, with the following paragraph:

As used herein, antibody fragment refers to any derivative of an antibody that is less [then] than full length, retaining at least a portion of the full-length antibody's specific binding ability. Examples of antibody fragments include, but are not limited to, Fab, Fab', $F(ab)_2$, single-chain Fvs (scFV), FV, dsFV diabody and Fd fragments. The fragment can include multiple chains linked together, such as by disulfide bridges. An antibody fragment generally contains at least about 50 amino acids and typically at least 200 amino acids.

Please amend the paragraph on page 34, lines 19-21, as follows:

As used herein, Fab [fragments] fragment is an antibody fragment that results from digestion of an immunoglobulin with papain; it can be recombinantly produced to produce the equivalent fragment.

Please amend the paragraph on page 37, lines 21-25, as follows:

As used herein, inhibitor of an activity of an MTSP encompasses any substances that prohibit or decrease production, post-translational modification(s), maturation, or membrane localization of the MTSP or any substances that interfere with or decrease the proteolytic efficacy of thereof, particularly of a single chain form *in vitro* for [screeing] screening assay.

Please amend the paragraph on page 39, lines 3-18, as follows:

As used herein, a peptidomimetic is a compound that mimics the conformation and certain stereochemical features of the biologically active form of a particular peptide. In general, peptidomimetics are designed to mimic certain desirable properties of a compound, but not the undesirable properties, such as flexibility, that lead to a loss of a biologically active conformation and bond breakdown. Peptidomimetics may be prepared from biologically active compounds by replacing certain groups or bonds that contribute to the undesirable properties with bioisosteres. Bioisosteres are known to those of skill in the art. For example the methylene bioisostere CH_2S has been used as an amide replacement in enkephalin analogs (see, *e.g.*, Spatola (1983) pp. 267-357 in *Chemistry and Blochemistry of Amino Acids, Peptides, and Proteins,* Weistein, Ed. volume 7, Marcel Dekker, New York). Morphine, which can be administered orally, is a compound that is a peptidomimetic of the peptide endorphin. For purposes herein, cyclic peptides are included among [pepidomimetics] peptidomimetics.

Please amend the paragraph on page 40, lines 3-16, as follows:

As used herein, a receptor refers to a molecule that has an affinity for a given ligand. Receptors can be naturally-occurring or synthetic molecules. Receptors can also be referred to in the art as anti-ligands. As used herein, the receptor and anti-ligand are interchangeable. Receptors can be used in their unaltered state or as aggregates with other species. Receptors can be attached, covalently or noncovalently, or in physical contact with, to a binding member, either directly or indirectly via a specific binding substance or linker. Examples

of receptors, include, but are not limited to: antibodies, cell membrane [receptors] receptors, surface receptors and internalizing receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants (such as on viruses, cells, or other materials), drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles.

Please amend the paragraph on page 47, lines 4-15, as follows:

The MTSPs are a family of transmembrane serine proteases that are found in mammals and also other species that share a number of common structural features including: a proteolytic extracellular C-terminal domain; a transmembrane domain, with a hydrophobic domain near the N-terminus; a short cytoplasmic domain; and a variable length stem region containing modular domains. The proteolytic domains share sequence [homologoy] homology including conserved his, asp, and ser residues necessary for catalytic activity that are present in conserved motifs. The MTSPs are synthesized as zymogens, and activated to two chain forms by cleavage. It is shown herein that the single chain proteolytic domain can function *in vitro* and, hence is useful in *in vitro* assays for identifying agents that modulate the activity of members of this family.

Please amend the paragraph on page 50, line 9 through page 51, line 10, as follows:

In specific aspects, the MTSP protease domains, portions thereof, and muteins thereof are from or based on animal MTSPS, including, but are not limited to, rodent, such as mouse and rat; fowl, such as chicken; ruminants, such as goats, cows, deer, sheep; ovine, such as pigs; and humans. In particular, MTSP7 derivatives can be made by altering their sequences by substitutions, additions or deletions. Due to the degeneracy of nucleotide coding sequences, other nucleic sequences which encode substantially the same amino acid sequence as a MTSP7 gene can be used. These include but are not limited to nucleotide sequences comprising all or portions of MTSP7 genes that

are altered by the substitution of different codons that encode the amino acid residue within the sequence, thus producing a silent change. Likewise, the MTSP7 derivatives include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of MTSP7, including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence can be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid (see, *e.g.*, Table 1). Muteins of the MTSP7 or a domain thereof, such as a protease domain, in which up to about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90% or 95% of the amino acids are replaced with another amino acid are provided. Generally such muteins retain at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the protease activity <u>of</u> the unmutated protein.

Please amend the paragraph on page 53, lines 1-11, as follows:

Muteins can be made by making conservative amino acid [substitions] <u>substitutions</u> and also non-conservative amino acid substitutions. For example, amino acid substitutions [the] *that* desirably alter properties of the proteins can be made. In one embodiment, mutations that prevent degradation of the polypeptide can be made. Many proteases cleave after basic residues, such as R and [K;] <u>K,</u> to eliminate such [cleavage,] *cleavage;* the basic residue is replaced with a non-basic residue. Interaction of the protease with an inhibitor

can be blocked while retaining catalytic activity by effecting a non-conservative change at the site interaction of the inhibitor with the protease. Receptor binding can be altered without altering catalytic activity.

Please amend the paragraph on page 54, lines 12-26, as follows:

In certain embodiments, the isolated nucleic acid fragment hybridizes to the nucleic acid having the nucleotide sequence set forth in SEQ ID No: 15 (or the molecules in the [figure in the FIGURE] Figure 1) under high stringency conditions, and generally contains the sequence of nucleotides set forth in SEQ ID Nos. 15-17; see also the Figure 1). The protein contains a transmembrane domain (TM), a SEA domain and a serine protease domain. Muteins of the protein are also provided in which amino acids are replaced with conservative amino acids. Among the muteins are those in which the Cys residues, is/are replaced with generally conservative amino acid residues, such as a serine. Such muteins are also provided herein. Each of such domains is provided herein as are nucleic acid molecules that include sequences of nucleotides encoding such domains. Some MTSPs can additionally include a LDLR domain, a scavenger-receptor cysteine rich (SRCR) domain and other domains.

Please amend the paragraph on page 56, line 16, through page 57, line 2, as follows:

The isolated nucleic acids can include [of] at least 8 nucleotides of an MTSP7-encoding sequence. In other embodiments, the nucleic acids can contain at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of an MTSP7-encoding sequence, or a full-length MTSP coding sequence. In another embodiment, the nucleic acids are smaller than 35, 200 or 500 nucleotides in length. Nucleic acids can be single or double stranded. Nucleic acids that [hybridizes] hybridize to or are complementary to the foregoing sequences, in particular the inverse complement to nucleic acids that hybridizes to the foregoing sequences (*i.e.*, the inverse complement of a nucleic acid strand has the complementary sequence running in reverse orientation to the strand so that the inverse complement would

hybridize without mismatches to the nucleic acid strand; thus, for example, where the coding strand hybridizes to a nucleic acid with no mismatches between the coding strand and the hybridizing strand, then the inverse complement of the hybridizing strand is identical to the coding strand) are also provided.

Please amend the paragraph on page 57, lines 14-21, as follows:

Probes and primers derived from the nucleic acid molecules are [provided,] provided. Such probes and primers contain at least 8, 14, 16, 30, 100 or more contiguous nucleotides with identity to contiguous nucleotides of an MTSP7, generally except for nucleic acids encoding 117-171 and 185-354 of SEQ ID No. 15. The probes and primers are optionally labelled with a detectable label, such as a radiolabel or a fluorescent tag, or can be mass differentiated for detection by mass spectrometry or other means.

Please amend the paragraph on page 59, line 30, through page 60, line 14, as follows:

After successful amplification of the nucleic acid containing all or a portion of the identified MTSP protein sequence or of a nucleic acid encoding all or a portion of an MTSP protein homolog, that segment can be molecularly cloned and sequenced, and used as a probe to isolate a complete cDNA or genomic clone. This, in turn, permits the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis. Once the nucleotide sequence is determined, an open reading frame encoding the MTSP protein gene protein product can be determined by any method well known in the art for determining open reading frames, for example, using publicly available computer programs for nucleotide sequence analysis. Once an open reading frame is defined, it is routine to determine the amino acid sequence of the protein encoded by the open reading frame. In this way, the nucleotide sequences of the entire MTSP protein genes as well as the amino acid sequences of MTSP [protein] proteins and analogs can be identified.

Please amend the paragraph on page 64, lines 1-15, as follows:

In one embodiment, the vectors include a sequence of nucleotides that encodes a polypeptide that has protease activity and contains all or a portion of only the protease domain, or multiple copies thereof, of an MTSP protein are provided. Also provided are vectors that comprise a sequence of nucleotides that encodes the protease domain and additional portions of an MTSP protein up to and including a full length MTSP protein, as well as multiple copies thereof [are also provided]. The vectors can be selected for expression of the MTSP protein or protease domain thereof in the cell or such that the MTSP protein is expressed as a transmembrane protein. Alternatively, the vectors can include signals necessary for secretion of encoded proteins. When the protease domain is [expressed] expressed, the nucleic acid can be linked to a nucleic acid sequence encoding a secretion signal, such as the *Saccharomyces cerevisiae α* mating factor signal sequence or a portion thereof sufficient for secretion. Any such signal sequence can be used.

Please amend the paragraph on page 67, line 25, through page 68, line 8, as follows:

The MTSP domains, derivatives and analogs can be produced by various methods known in the art. For example, once a recombinant cell expressing an MTSP protein, or a domain, fragment or derivative thereof, is identified, the individual gene product can be isolated and analyzed. This is achieved by assays based on the physical and/or functional properties of the protein, including, but not limited to, radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled product. The MTSP proteins can be isolated and purified by standard methods known in the art (either from natural sources or recombinant host cells expressing the complexes or proteins), including but not restricted to column chromatography (*e.g.*, ion exchange, affinity, gel exclusion, reversed-phase high pressure and fast protein liquid), differential centrifugation, differential solubility, or by any other standard technique used for the purification of proteins.

Functional properties can be evaluated using any suitable assay known in the art.

Please amend the paragraph on page 68, line 27, through page 69, line 11, as follows:

In addition, domains, analogs and derivatives of an MTSP protein can be chemically synthesized. For example, a peptide corresponding to a portion of an MTSP protein, which includes the desired domain or which mediates the desired activity *in vitro* can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the MTSP protein sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, [a] $\underline{\alpha}$-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, ϵ-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionoic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, *t*-butylglycine, *t*-butylalanine, phenylglycine, cyclohexylalanine, ß-alanine, fluoro-amino acids, designer amino acids such as ß-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Please amend the paragraph on page 71, lines 12-27, as follows:

In addition, domains, analogs and derivatives of a MTSP can be chemically synthesized. For example, a peptide corresponding to a portion of a MTSP, which comprises the desired domain or which mediates the desired activity *in vitro* can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the MTSP sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, [a] $\underline{\alpha}$-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-aminobutyric acid, ϵ-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionoic acid, ornithine, norleucine, norvaline,

hydroxyproline, sarcosine, citrulline, cysteic acid, *t*-butylglycine, *t*-butylalanine, phenylglycine, cyclohexylalanine, ß-alanine, fluoro-amino acids, designer amino acids such as ß-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Please amend the paragraph on page 71, line 29, through page 72, line 5, as follows:

The single chain protease domains, as shown herein, can be used in a variety of methods to identify compounds that modulate the activity thereof. For MTSPs that exhibit higher activity or expression in tumor cells, compounds that inhibit the proteolytic activity are of particular interest. For any MTSPs that are active at lower levels in tumor cells, compounds or agents that enhance the activity are potentially of interest. In all instances the identified compounds will include agents that are [candidate] candidates for cancer treatments.

Please amend the paragraph on page 78, lines 14-15, as follows:

4. Methods for Identifying Agents that Modulate the Expression of a Nucleic Acid Encoding an MTSP7

Please amend the paragraph on page 85, lines 3-6, as follows:

The compounds identified by the screening methods include inhibitors, including antagonists, and can be [agonists] agonists. Compounds for screening are any compounds and collections of compounds available, know or that can be prepared.

Please amend the paragraph on page 86, line 27, through page 87, line 8, as follows:

To evaluate the ability of a compound to reduce the occurrence of, or inhibit, metastasis, the procedures described by Kobayashi et al., *Int. J. Canc.*, 57:727-733d (1994) can be employed. Briefly, a murein xenograft selected for high lung colonization potential [in] is injected into C57B1/6 mice i.v. (experimental metastasis) or s.c. into the abdominal wall (spontaneous metastasis). Various concentrations of the compound to be tested can be

admixed with the tumor cells in Matrigel prior to injection. Daily i.p. injections of the test compound are made either on days 1-6 or days 7-13 after tumor inoculation. The animals are sacrificed about three or four weeks after tumor inoculation, and the lung tumor colonies are counted. Evaluation of the resulting data permits a determination as to efficacy of the test compound, optimal dosing and route of administration.

Please amend the paragraph on page 88, lines 19-24, as follows:

In vivo experimental [modes] models designed to evaluate the inhibitory potential of a test serine protease [inhibitors] inhibitor, using a tumor cell line F3II, [the] found to be highly invasive, are described by Alonso et al., *Breast Canc. Res. Treat.*, 40:209-223 (1996). This group describes *in vivo* studies for toxicity determination, tumor growth, invasiveness, spontaneous metastasis, experimental lung metastasis, and an angiogenesis assay.

Please amend the paragraph on page 89, line 26, through page 93, line 8, as follows:

Exemplary, but not limiting serine proteases, include the following known serine protease inhibitors are used in the screening assays: Serine Protease Inhibitor 3 (SPI-3) (Chen, M.C., et al., *Citokine, 11(11)*:856-862 (1999)); Aprotinin (Iijima, R., et al., *J. Biochem. (Tokyo), 126(5)*:912-916 (1999)); Kazal-type serine protease inhibitor-like proteins (Niimi, T., et al., *Eur. J. Biochem., 266(1)*:282-292 (1999)); Kunitz-type serine protease inhibitor (Ravichandran, S., et al., *Acta Crystallogr. D. Biol. Crystallogr., 55(11)*:1814-1821 (1999)); Tissue factor pathway inhibitor-2/Matrix-associated serine [rotease] protease inhibitor (TFPI-2/MSPI), (Liu, Y., et al., *Arch. Biochem. Biophys., 370(1)*:112-8 (1999)); Bukunin, (Cui, C.Y., et al., *J. Invest. Dermatol., 113(2)*:182-8 (1999)); Nafmostat mesilate (Ryo, R., et al., *Vox Sang., 76(4)*:241-6 (1999)); TPCK (Huang, Y., et al., *Oncogene, 18(23)*:3431-9 (1999)); A synthetic cotton-bound serine protease inhibitor (Edwards, J.V., et al., *Wound Repair Regen., 7(2)*:106-18 (1999)); FUT-175 (Sawada, M., et al., *Stroke, 30(3)*:644-50 (1999)); Combination of serine protease inhibitor FUT-0175 and thromboxane synthetase

inhibitor OKY-046 (Kaminogo, M., et al., *Neurol. Med. Chir. (Tokyo), 38(11)*:704-8; discussion 708-9 (1998)); The rat serine protease inhibitor 2.1 gene (LeCam, A., et al., *Biochem. Biophys. Res. Commun., 253(2)*:311-4 (1998)); A new intracellular serine protease inhibitor expressed in the rat pituitary gland complexes with granzyme B (Hill, R.M., et al., *FEBS Lett., 440(3)*:361-4 (1998)); 3,4-Dichloroisocoumarin (Hammed, A., et al., *Proc. Soc. Exp. Biol. Med., 219(2)*:132-7 (1998)); LEX032 (Bains, A.S., et al., *Eur. J. Pharmacol., 356(1)*:67-72 (1998)); N-tosyl-L-phenylalanine chloromethyl ketone (Dryjanski, M., et al., *Biochemistry, 37(40)*:14151-6 (1998)); Mouse gene for the serine protease inhibitor neuroserpin (P112) (Berger, P., et al., *Gene, 214(1-2)*:25-33 (1998)); Rat serine protease inhibitor 2.3 gene (Paul, C., et al., *Eur. J. Biochem., 254(3)*:538-46 (1998)); Ecotin (Yang, S.Q., et al., *J. Mol. Biol., 279(4)*:945-57 (1998)); A 14 kDa plant-related serine protease inhibitor (Roch, P., et al., *Dev. Comp. Immunol., 22(1)*:1-12 (1998)); Matrix-associated serine protease inhibitor TFPI-2/33 kDa MSPI (Rao, C.N., et al., *Int. J. Cancer, 76(5)*:749-56 (1998)); ONO-3403 (Hiwasa, T., et al., *Cancer Lett., 126(2)*:221-5 (1998)); Bdellastasin (Moser, M., et al., *Eur. J. Biochem., 253(1)*:212-20 (1998)); Bikunin (Xu, Y., et al., *J. Mol. Biol., 276(5)*:955-66 (1998)); Nafamostat mesilate (Mellgren, K., et al., *Thromb. Haemost., 79(2)*:342-7 (1998)); The growth hormone dependent serine protease inhibitor, Spi 2.1 (Maake, C., et al., *Endocrinology, 138(12)*:5630-6 (1997)); Growth factor activator inhibitor type 2, a Kunitz-type serine protease inhibitor (Kawaguchi, T., et al., *J. Biol. Chem., 272(44)*:27558-64 (1997)); Heat-stable serine protease inhibitor protein from ovaries of the desert locust, Schistocerga gregaria (Hamdaoui, A., et al., *Biochem. Biophys. Res. Commun., 238(2)*:357-60 (1997)); Bikunin, (Delaria, K.A., et al., *J. Biol. Chem., 272(18)*:12209-14 (1997)); Human placental bikunin (Marlor, C.W., et al., *J. Biol. Chem., 272(10)*:12202-8 (1997)); Hepatocyte growth factor activator inhibitor, a novel Kunitz-type serine protease inhibitor (Shimomura, T., et al., *J. Biol. Chem., 272(10)*:6370-6 (1997)); FUT-187, oral serine protease inhibitor, (Shiozaki, H.,

et al., *Gan To Kaguku Ryoho, 23(14)*: 1971-9 (1996)); Extracellular matrix-associated serine protease inhibitors (Mr 33,000, 31,000, and 27,000 (Rao, C.N., et al., *Arch. Biochem. Biophys., 335(1)*:82-92 (1996)); An irreversible isocoumarin serine protease inhibitor (Palencia, D.D., et al., *Biol. Reprod., 55(3)*:536-42 (1996)); 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF) (Nakabo, Y., et al., *J. Leukoc. Biol., 60(3)*:328-36 (1996)); Neuroserpin (Osterwalder, T., et al., *EMBO J., 15(12)*:2944-53 (1996)); Human serine protease inhibitor alpha-1-antitrypsin (Forney, J.R., et al., *J. Parasitol. 82(3)*:496-502 (1996)); Rat serine protease inhibitor 2.3 (Simar-Blanchet, A.E., et al., *Eur. J. Biochem., 236(2)*:638-48 (1996)); Gebaxate mesilate (parodi, F., et al., *J. Cardiothorac. Vasc. Anesth., 10(2)*:235-7 (1996)); Recombinant serine protease inhibitor, CPTI II (Stankiewicz, M., et al., (*Acta Biochim. Pol., 43(3)*:525-9 (1996)); A cysteine-rich serine protease inhibitor (Guamerin II) (Kim, D.R., et al., *J. Enzym. Inhib., 10(2)*:81-91 (1996)); Diisopropylfluorophosphate (Lundqvist, H., et al., *Inflamm. Res., 44(12)*:510-7 (1995)); Nexin 1 (Yu, D.W., et al., *J. Cell Sci., 108(Pt 12)*:3867-74 (1995)); LEX032 (Scalia, R., et al., *Shock, 4(4)*:251-6 (1995)); Protease nexin I (Houenou, L.J., et al., *Proc. Natl. Acad. Sci. U.S.A., 92(3)*:895-9 (1995)); Chymase-directed serine protease inhibitor (Woodard S.L., et al., *J. Immunol., 153(11)*:5016-25 (1994)); N-alpha-tosyl-L-lysyl-chloromethyl ketone (TLCK) (Bourinbaiar, A.S., et al., *Cell Immunol., 155(1)*:230-6 (1994)); Smpi56 (Ghendler, Y., et al., *Exp. Parasitol., 78(2)*:121-31 (1994)); Schistosoma haematobium serine protease (Blanton, R.E., et al., *Mol. Biochem. Parasitol., 63(1)*:1-11 (1994)); Spi-1 (Warren, W.C., et al., *Mol. Cell Endocrinol., 98(1)*:27-32 (1993)); TAME (Jessop, J.J., et al., *Inflammation, 17(5)*:613-31 (1993)); Antithrombin III (Kalaria, R.N., et al., *Am. J. Pathol., 143(3)*:886-93 (1993)); FOY-305 (Ohkoshi, M., et al., *Anticancer Res., 13(4)*:963-6 (1993)); Camostat mesilate (Senda, S., et al., *Intern. Med., 32(4)*:350-4 (1993)); Pigment epithelium-derived factor (Steele, F.R., et al., *Proc. Natl. Acad. Sci. U.S.A., 90(4)*:1526-30 (1993)); Antistasin (Holstein, T.W., et al., *FEBS Lett.*,

309(3):288-92 (1992)); The vaccinia virus K2L gene encodes a serine protease inhibitor (Zhou, J., et al., *Virology, 189(2)*:678-86 (1992)); Bowman-Birk serine-protease inhibitor (Werner, M.H., et al., *J. Mol. Biol., 225(3)*:873-89 (1992); FUT-175 (Yanamoto, H., et al., *Neurosurgery, 30(3)*:358-63 (1992)); FUT-175; (Yanamoto, H., et al., *Neurosurgery, 30(3)*:351-6, discussion 356-7 (1992)); PAI-I (Yreadwell, B.V., et al., *J. Orthop. Res., 9(3)*:309-16 (1991)); 3,4-Dichloroisocoumarin (Rusbridge, N.M., et al., *FEBS Lett., 268(1)*:133-6 (1990)); Alpha 1-antichymotrypsin (Lindmark, B.E., et al., *Am. Rev. Respir. Des., 141(4 Pt 1)*:884-8 (1990)); P-toluenesulfonyl-L-arginine methyl ester (TAME) (Scuderi, P., *J. Immunol., 143(1)*:168-73 (1989)); Aprotinin (Seto, S., et al., *Adv. Exp. Med. Biol., 247B*:49-54 (1989)); Alpha 1-antichymotrypsin (Abraham, C.R., et al., *Cell, 52(4)*:487-501 (1988)); Contrapsin (Modha, J., et al., *Parasitology, 96 (Pt 1)*:99-109 (1988)); (FOY-305) (Yamauchi, Y., et al., *Hiroshima J. Med. Sci., 36(1)*:81-7 No abstract available (1987)); Alpha 2-antiplasmin (Holmes, W.E., et al., *J. Biol. Chem., 262(4)*:1659-64 (1987)); 3,4-dichloroisocoumarin (Harper, J.W., et al., *Biochemistry, 24(8)*:1831-41 (1985)); Diisoprophylfluorophosphate (Tsutsui, K., et al., *Biochem. Biophys. Res. Commun., 123(1)*:271-7 (1984)); Gabexate mesilate (Hesse, B., et al., *Pharmacol. Res. Commun., 16(7)*:637-45 (1984)); Phenyl methyl sulfonyl fluoride (Dufer, J., et al., *Scand. J. Haematol., 32(1)*:25-32 (1984)); Aprotinin (Seto, S., et al., *Hypertension, 5(6)*:893-9 (1983)); Protease inhibitor CI-2 (McPhalen, C.A., et al., *J. Mol. Biol., 168(2)*:445-7 (1983)); Phenylmethylsulfonyl fluoride (Sekar V., et al., *Biochem. Biophys. Res. Commun., 89(2)*:474-8 (1979)); PGE1 (Feinstein, M.D., et al., *Prostaglandine, 14(6)*:1075-93 (1977)

Please amend the paragraph on page 94, lines 1-17, as follows:

The libraries fall into roughly three categories: fusion-protein-displayed peptide libraries in which random peptides or proteins are presented on the surface of phage particles or proteins expressed from plasmids; support-bound synthetic chemical libraries in which individual compounds or mixtures of compounds are presented on insoluble matrices, such as resin beads (*see, e.g.*,

Lam et al., *Nature*, *354*:82-84 (1991)) and cotton supports (*see, e.g.*, Eichler et al., *Biochemistry 32*:11035-11041 (1993)); and methods in which the compounds are used in solution (*see, e.g.*, Houghten et al., *Nature*, *354*:84-86 (1991); Houghten et al., *BioTechniques*, *313*:412-421 (1992); and Scott et al., *Curr. Opin. Biotechnol.*, *5*:40-48 (1994)). There are numerous examples of synthetic peptide and oligonucleotide combinatorial libraries, and there are many methods for producing libraries that contain non-peptidic small organic molecules. Such libraries can be based on a [basis] basic set of monomers that are combined to form mixtures of diverse organic molecules or that can be combined to form a library based upon a selected pharmacophore monomer.

Please amend the paragraph on page 99, lines 11-19, as follows:

Provided herein are compounds, identified by screening or produced using the MTSP7 protein or protease domain in other screening methods, that modulate the activity of an MTSP7. These compounds act by directly interacting with the MTSP7 protein or by altering transcription or translation thereof. Such molecules include, but are not limited to, antibodies that specifically react with an MTSP7 protein, particularly with the protease domain thereof, antisense nucleic acids that alter expression of the MTSP7 protein or dsRNA, such as [RNAi,,] RNAi, antibodies, peptide mimetics and other such compounds.

Please amend the paragraph on page 102, lines 12-26, as follows:

Provided herein are methods for identifying molecules that bind to and modulate the activity of MTSP proteins. Included among molecules that bind to MTSP7, particularly the single chain protease domain or catalytically active fragments thereof, are peptides, polypeptides and peptide mimetics, including cyclic peptides. Peptide mimetics are molecules or compounds that mimic the necessary molecular conformation of a ligand or polypeptide for specific binding to a target molecule such as an MTSP7 protein. In an exemplary embodiment, the [peptides] peptides, polypeptides and peptide mimetics [or peptide mimetics] bind to the protease domain of the MTSP7 protein. Such peptides and peptide

mimetics include those of antibodies that specifically bind an MTSP7 protein and, typically, bind to the protease domain of an MTSP7 protein. The peptides, polypeptides and peptide mimetics [and peptide mimetics] identified by methods provided herein can be agonists or antagonists of MTSP7 proteins.

Please amend the paragraph on page 103, line 29, through page 104, line 9, as follows:

Accordingly, the peptides, polypeptides and peptide mimetics that bind to an MTSP7 protein can be used <u>for</u> generating pharmaceutical compositions containing, as an active ingredient, at least one of the peptides, polypeptides or peptide mimetics in association with a pharmaceutical carrier or diluent. The compounds can be administered, for example, by oral, pulmonary, parental (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration (see, *e.g.*, International PCT application Nos. WO 93/25221 and WO 94/17784; and European Patent Application 613,683).

Please amend the paragraph on page 105, lines 4-17, as follows:

Moreover, based on their ability to bind to an MTSP7 protein, the peptides, polypeptides and peptide mimetics can be used as reagents for detecting MTSP7 proteins in living cells, fixed cells, in biological fluids, in tissue homogenates and in purified, natural biological materials. For example, by [labelling] <u>labeling</u> such peptides, polypeptides and peptide mimetics, cells having MTSP7 proteins can be indentified. In addition, based on their ability to bind an MTSP7 protein, the peptides, polypeptides and peptide mimetics can be used in *in situ* staining, FACS (fluorescence-activated cell sorting), Western blotting, ELISA and other analytical protocols. Based on their ability to bind to an MTSP7 protein, the peptides, polypeptides and peptide mimetics can be used in purification of MTSP7 protein polypeptides or in purifying cells expressing the

MTSP7 protein polypeptides, *e.g.*, a polypeptide encoding the protease domain of an MTSP7 protein.

Please amend the paragraph on page 112, lines 15-26, as follows:

Using the "encoded synthetic library" or "very large scale immobilized polymer synthesis" systems (see, *e.g.*, U.S. Patent No. 5,925,525, and 5,902,723); the minimum size of a peptide with the activity of interest can be determined. In addition all peptides that form the group of peptides that differ from the desired motif (or the minimum size of that motif) in one, two, or more residues can be prepared. This collection of peptides then can be screened <u>for their</u> ability to bind to the target molecule, *e.g.*, and MTSP7 protein or, generally, the protease domain of an MTSP7 protein. This immobilized polymer synthesis system or other peptide synthesis methods can also be used to synthesize truncation analogs and deletion analogs and combinations of truncation and deletion analogs of the peptide compounds.

Please amend the paragraph on page 116, lines 22-28, as follows:

These conjugates are used in a variety of methods and are particularly suited for use in methods of activation of prodrugs, such as prodrugs that, upon cleavage by the particular MTSP7 protein are cytotoxic. The prodrugs are administered prior [to] <u>to,</u> simultaneously with or subsequently to the conjugate. Upon delivery to the targeted cells, the protease activates the prodrug, which then exhibits [is] <u>its</u> therapeutic effect, such as a cytotoxic effect.

Please amend the paragraph on page 118, lines 5-24, as follows:

Linkers can be any moiety suitable to associate a domain of MTSP7 protein and a targeting agent. Such linkers and linkages include, but are not limited to, peptidic linkages, amino acid and peptide linkages, typically containing between one and about 60 amino acids, more generally between about 10 and 30 amino acids, chemical linkers, such as heterobifunctional cleavable cross-linkers, including but are not limited to, N-succinimidyl (4-iodoacetyl)-aminobenzoate, [sulfosuccinimydil (4-iodoacetyl)-aminobenzoate] <u>sulfosuccinimidyl (4-iodoacetyl)-aminobenzoate</u>, 4-succinimidyl-oxycarbonyl-a-

(2-pyridyldithio)toluene, [sulfosuccinimidyl-6- [a-methyl-a-(pyridyldithiol)-toluamido] hexanoate] sulfosuccinimidyl-6- [*α*-methyl-*α*-(pyridyldithiol)-toluamido] hexanoate, [N-succinimidyl-3-(-2-pyridyldithio) - proprionate] N-succinimidyl-3-(-2-pyridyldithio) - propionate, [succinimidyl 6[3(-(-2-pyridyldithio)-proprionamido] hexanoate] succinimidyl 6[3(-(-2-pyridyldithio)-propionamido] hexanoate, sulfosuccinimidyl 6[3(-(-2-pyridyldithio)-propionamido] hexanoate, 3-(2-pyridyldithio)-propionyl hydrazide, Ellman's reagent, dichlorotriazinic acid, and S-(2-thiopyridyl)-L-cysteine. Other linkers include, but are not limited to peptides and other moieties that reduce [stearic] steric hindrance between the domain of MTSP7 protein and the targeting agent, intracellular enzyme substrates, linkers that increase the flexibility of the conjugate, linkers that increase the solubility of the conjugate, linkers that increase the serum stability of the conjugate, photocleavable linkers and acid cleavable linkers.

Please amend the paragraph on page 121, line 26, through page 122, line 3, as follows:

Any agent that facilitates detection, immobilization, or purification of the conjugate is contemplated for use herein. For chemical conjugates any moiety that has such properties is contemplated; for fusion proteins, the targeting agent is a protein, peptide or fragment thereof that is sufficient to [effects] effect the targeting activity. Generally targeting agents are those that deliver the MTSP7 protein or portion thereof to selected cells and tissues. Such agents include tumor specific monoclonal antibodies and portions thereof, growth factors, such as FGF, EGF, PDGF, VEGF, cytokines, including chemokines, and other such agents.

Please amend the paragraph on page 122, lines 12-18, as follows:

Plasmids for replication and vectors for expression that contain the above nucleic acid fragments are also provided. Cells containing the plasmids and vectors are also provided. The cells can be any suitable host including, but are not limited to, bacterial cells, yeast cells, fungal cells, plant cells, insect [cell] cells and animal cells. The nucleic acids, plasmids, and cells containing the

plasmids can be prepared according to methods known in the art including any described herein.

Please amend the paragraph on page 122, lines 19-28, as follows:

Also provided are methods for producing the above fusion proteins. An exemplary method includes the steps of growing, i.e. culturing the cells so that [the] they proliferate, cells containing a plasmid encoding the fusion protein under conditions whereby the fusion protein is expressed by the cell, and recovering the expressed fusion protein. Methods for expressing and recovering recombinant proteins are well known in the art (*See generally, Current Protocols in Molecular Biology* (1998) § 16, John Wiley & Sons, Inc.) and such methods can be used for expressing and recovering the expressed fusion proteins. Typically, the recombinant expression and recovery methods described herein can be used.

Please amend the paragraph on page 123, line 28, through page 124, line 9, as follows:

The matrix material or solid supports contemplated herein are generally any of the insoluble materials known to those of skill in the art to immobilize ligands and other molecules, and are those that are used in many chemical syntheses and separations. Such supports are used, for example, in affinity chromatography, in the immobilization of biologically active materials, and during chemical syntheses of biomolecules, including proteins, amino acids and other organic molecules and polymers. The preparation of and use of supports is well known to those of skill in this art; there are many such materials and preparations thereof known. For example, naturally-occurring support materials, such as agarose and cellulose, can be isolated from their respective sources, and processed according to known protocols, and synthetic materials can be prepared in accord with known protocols.

Please amend the paragraph on page 129, line 29, through page 130, line 4, as follows:

The MTSP7 protein modulator and the anti-tumor agent can be packaged as separate compositions for administration together or sequentially or intermittently. Alternatively, they can be provided as a single composition for administration or as two compositions for administration as a single composition. The combinations can be packaged as kits.

Please amend the paragraph on page 131, lines 2-4, as follows:

The compounds identified by the methods provided herein [or provided herein] can be used in combination with anti-tumor agents and/or anti-angiogenesis agents.

Please amend the paragraph on page 134, line 19, through page 135, line 16, as follows:

The compounds can also be mixed with other active materials, that do not impair the desired action, or with materials that supplement the desired action known to those of skill in the art. The formulations of the compounds and agents for use herein include those suitable for oral, rectal, topical, inhalational, buccal (*e.g.*, sublingual), parenteral (*e.g.*, subcutaneous, intramuscular, intradermal, or intravenous), transdermal administration or any route. The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active compound which is being used. The formulations are provided for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, and oral solutions or suspensions, and oil-water emulsions containing suitable quantities of the compounds or pharmaceutically acceptable derivatives thereof. The pharmaceutically therapeutically active compounds and derivatives thereof are typically formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal subjects and packaged individually

as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include [ampoules] ampules and syringes and individually packaged tablets or capsules. Unit-dose forms can be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles of tablets or capsules or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.

Please amend the paragraph on page 147, lines 5-20, as follows:

In a specific embodiment, a viral vector that contains the MTSP7 protein nucleic acid is used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol. 217*:581-599 (1993)). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The MTSP7 protein nucleic acid to be used in gene therapy is cloned into the vector, which facilitates delivery of the gene into a patient. More [detail] details about retroviral vectors can be found in Boesen et al., *Biotherapy 6*:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest. 93*:644-651 (1994); Kiem et al., *Blood 83*:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy 4*:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel. 3*:110-114 (1993).

Please amend the paragraph on page 151, lines 2-15, as follows:

A method for treating tumors is provided. The method is practiced by administering a prodrug that is specifically cleaved by an MTSP7 to release an active drug. Upon contact with a cell that expresses MTSP7 activity, the prodrug is converted into an active drug. The prodrug can be a conjugate that

contains the active agent, such as an anti-tumor drug, such as a cytotoxic agent, or other therapeutic agent, [linked,] linked to a substrate for the targeted MTSP7, such that the drug or agent is inactive or unable to enter a cell, in the conjugate, but is activated upon cleavage. The prodrug, for example, can contain an oligopeptide, typically a relatively short, less than about 10 amino acids peptide, that is selectively proteolytically cleaved by the targeted MTSP7. Cytotoxic agents, include, but are not limited to, alkylating agents, antiproliferative agents and tubulin binding agents. Others include, vinca drugs, mitomycins, bleomycins and taxanes.

Please amend the paragraph on page 151, line 17, through page 152, line 2, as follows:

Transgenic animal models are provided herein. Such an animal can [by] <u>be</u> produced by promoting recombination between an exogenous MTSP7 gene that could be over-expressed or mis-expressed, such as by expression under a strong promoter, via homologous or other recombination event. For example, transgenic animals can be produced by introducing the nucleic acid using vectors or other modes of gene delivery into a germline cell, such as an embryonic stem cell. Typically the nucleic acid is introduced, such as an embryonic stem cell, which is then injected by transforming embryo-derived stem (ES) cells with a vector containing the MTSP7 protein-encoding nucleic acid followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of a transgenic animal. Generally introduction into a chromosome of the animal occurs by a recombination between the heterologous MTSP7-encoding nucleic acid and endogenous nucleic acid. The heterologous nucleic acid can be targeted to a specific chromosome.

Please amend the paragraph on page 160, lines 6-25, as follows:

After a 5L fermentation, cells were separated from the medium by centrifugation. The supernatant was brought to 40% saturation with ammonium sulfate and centrifuged. The pellet was dissolved in 250 mL 20 mM

Hepes, 150 mM NaCl pH 7.5 (HS buffer). The resulting protein solution was stored at [–20i° C n] –20°C in 50 mL aliquots. After thawing, 50 mL of protein solution was dialyzed against 4 L of HS buffer. To the dialysate, 4 mL of 10 % polyethylenimine was added, and the precipitate was removed by centrifugation at 20,000 rpm. The supernatant was diluted with HS buffer to a volume of 90 mL and 20 g of ammonium sulfate was added slowly. The resulting solution was applied onto an octyl sepharose column (2.5 cm x 10 cm) (Pharmacia) equilibrated with HS buffer containing 1.74 M ammonium sulfate at room temperature. After washing the column,with HS buffer containing 1.74 M ammonium sulfate, MTSP7 was eluted in a gradient (1.74 M – 0 ammonium sulfate in HS buffer). Active fractions were pooled (60 mL) and dialyzed against HS buffer overnight with one buffer change. The dialysate was batch-absorbed onto 20 mL benzamidine sepharose (Pharmacia) and washed with 4 column volumes HS buffer. MTSP 7 was eluted by HS/4 mM benzamidine. The protein appeared homogeneous by SDS-PAGE, and the identity of the protein was confirmed by amino acid sequencing.

Please amend the paragraph on page 160, lines 27-28, as follows:

Assays for identification of candidate compounds that modulate [that] the activity of an MTSP

Please amend the paragraph on page 162, line 26, through page 163, line 2, as follows:

The ability of a [compounds] compound to act as a selective inhibitor of matriptase activity was assessed by determining the concentration of test compound that inhibits the activity of matriptase by 50%, (IC_{50}) as described in the above Example, and comparing IC_{50} value for matriptase to that determined for all or some of the following serine proteases: thrombin, recombinant tissue plasminogen activator (rt-PA), plasmin, activated protein C, chymotrypsin, factor Xa and trypsin.

In the Claims:

Please amend claims 13, 23, 79, 109, 112 and 115 as follows:

13. (Amended) The polypeptide of claim 12, wherein the domain is the protease [domain] domain.

23. (Amended) An isolated nucleic acid molecule that encodes a mutein of claim 15.

79. (Amended) A method for identifying activators of the zymogen form of an MTSP7, comprising:

contacting a zymogen form of the polypeptide of claim 1 with a substrate of the activated form of the polypeptide;

[add] adding a test compound, wherein the test compound is added before, after or simultaneously with the addition of the substrate; and

detecting cleavage of the substrate, thereby identifying compounds that activate the zymogen.

109. (Amended) The method of claim 108, wherein the tumor [is tumor] is a tumor of the breast, cervix, prostate, lung, ovary or colon.

112. (Amended) The method of claim 111, wherein the tumor [is tumor] is a tumor of the breast, cervix, prostate, lung, ovary or colon.

115. (Amended) The method of claim 114, wherein the tumor [is tumor] is a tumor of the breast, cervix, prostate, lung, ovary or colon.